Separation of photo-induced radical pair in cryptochrome to a functionally critical distance (Supplementary information)

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December 22, 2013

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In vacuo model of electron transfer chain in cryptochrome

The *in vacuo* model for studying electron transfer in the isoalloxazine moiety and the tryptophan triad complex is depicted in Fig. S1a. This model neglects the influence of the protein matrix other than the tryptophan triad on the electron transfer in cryptochrome, and, therefore, comparison with the model introduced in Fig. 2, that includes the protein environment, permits one to quantify the protein influence on the excitation energies.

The quantum chemical description of the *in vacuo* model included the redox states of the cryptochrome active site characterized through (i) the oxidized flavin, FAD, with neutral (reduced) W377 and W400 residues, (ii) the radical pair [$FAD^{\bullet-}$ + $W400^{\bullet+}$] with reduced W377 and (iii) the radical pair [$FAD^{\bullet-}$ + $W377^{\bullet+}$] with reduced W400. The electronic states of the *in vacuo* model of the cryptochrome active site are shown in Fig. S1b-c. The geometry optimization was performed with the state-averaged CASSCF(8,7) method employing the protocol developed earlier[1,2], assuming equal weights for all states considered (Fig. S1b). At the optimized geometries, the excitation spectra were computed using the perturbation theory-based XMCQDPT2 method[3] (Fig. S1c). The energy-optimized configurations are denoted in Fig. S1 as GS, RP1, and RP2. The electronic wavefunction was expanded in all calculations using the 6-31G* basis set.

Figure S1, along with Fig. 3, shows that the energy of the optimized [$FAD^{\bullet-}$ + $W400^{\bullet+}$] radical pair *in vacuo* (state of optimal geometry RP1) is 0.67 eV higher than the energy of this state optimized taking into account the cryptochrome environment as specified in Fig. 2. Remarkably, the energy of the optimized [$FAD^{\bullet-}$ + $W377^{\bullet+}$] radical pair *in vacuo* is 1.59 eV higher than in the cryptochrome environment, resulting for the *in vacuo* case in an energy difference $\Delta G_{vac} = 1.18$ eV between the RP1 and RP2 states. The reorganization energy λ_{vac} for the active site model *in vacuo* is also noticeably higher than when the cryptochrome is included, namely 1.93 eV. The high *in vacuo* values of ΔG_{vac} and λ_{vac} indicate that without

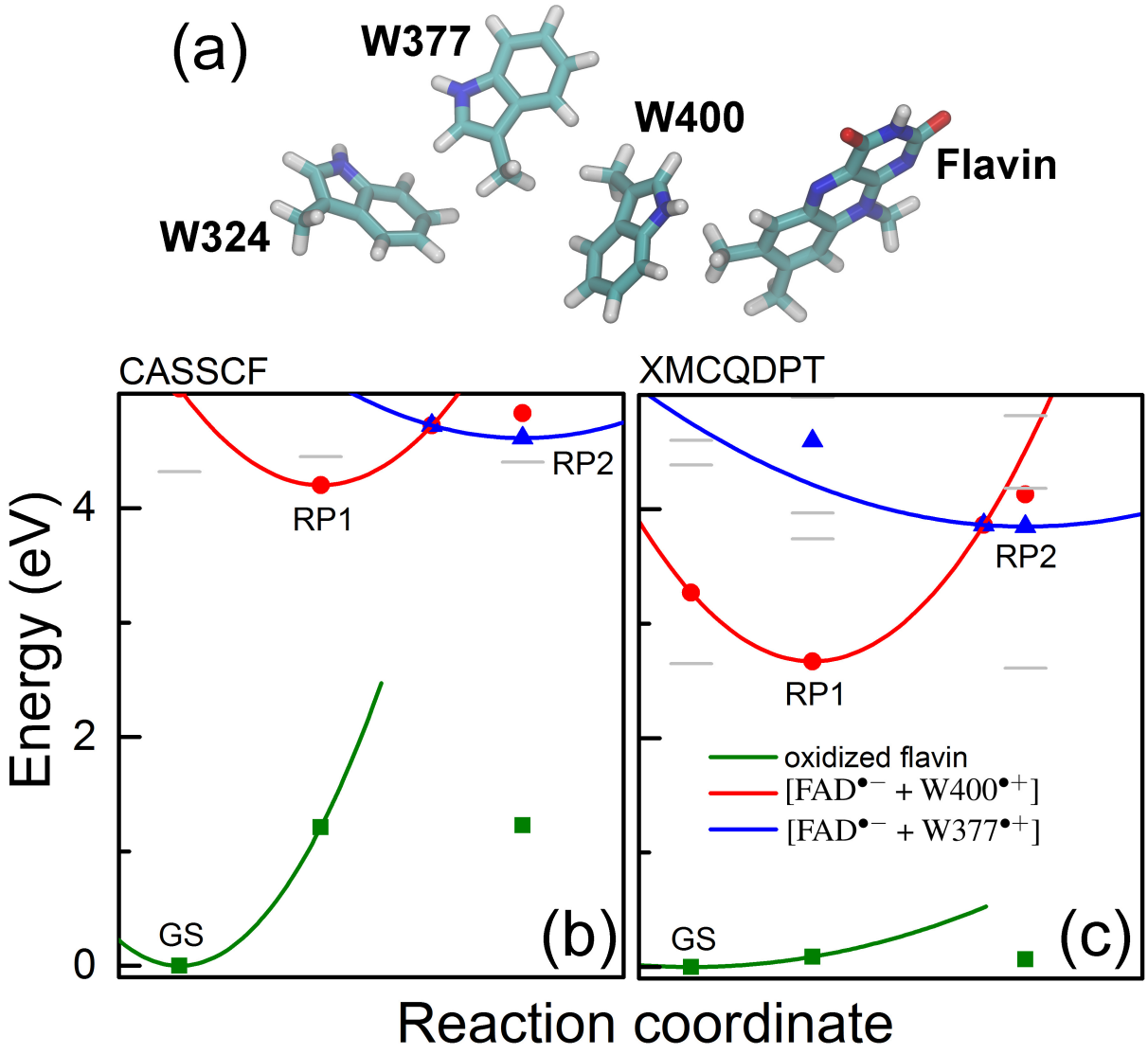

Figure S1: **Potential energy profiles of the key electronic states in the flavin - tryptophan triad electron transfer chain *in vacuo*.** (a) The *in vacuo* model includes the isoalloxazine moiety of FAD and the tryptophan triad W400, W377, and W324. (b) The energy for oxidized FAD is shown in green, for radical pair state [$FAD^{\bullet-}$+$W400^{\bullet+}$] in red and for radical pair state [$FAD^{\bullet-}$+$W377^{\bullet+}$] in blue. Symbols represent calculated energies, while lines represent schematic potential energy surfaces. Gray ticks show energies of the other excited states obtained in the calculations. The energies were computed using the CASSCF method. (c) Same as in (b), but energies computed using the perturbation theory-based XMCQDPT2 method.

the stabilizing interactions in cryptochrome the W377→ $W400^{\bullet+}$ electron transfer cannot occur fast enough to be relevant for cryptochrome signaling.

Figure S1 shows that *in vacuo* the two radical pair states RP1 and RP2, namely the geometry-optimized states for [$FAD^{\bullet-}$ + $W400^{\bullet+}$] and [$FAD^{\bullet-}$ + $W377^{\bullet+}$], respectively, are not the second and third excited states of the system, as it happened to be the case for the cryptochrome active site model shown in Fig. 2, but rather other types of redox states lie in between RP1 and RP2, such as flavin and tryptophan electronic excitations.

A comparison between the *in vacuo* results in Fig. S1 and the cryptochrome states in Fig. 3 reveals that the protein matrix surrounding the electron transfer chain impacts strongly the energy ordering of [$FAD^{\bullet-}$ + $W400^{\bullet+}$] and [$FAD^{\bullet-}$ + $W377^{\bullet+}$]. Accounting for all polar and charged amino acids within a 6 Å distance from flavin, W400 and W377 (see Fig. 2) in our quantum chemical description should reproduce to a large degree the influence of the protein on W377→ $W400^{\bullet+}$ electron transfer and settle the question which step proceeds faster, D396 facilitated $W400^{\bullet+}$→$FAD^{\bullet-}$ proton transfer or W377→ $W400^{\bullet+}$ electron transfer.

References

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